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EFFECT OF SOLID STATE FERMENTATION (SSF) BIOMASS SUPPLEMENTATION ON DIGESTIBILITY, NUTRIENT UTILIZATION AND RUMEN FERMENTATION PATTERN IN SHEEP

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ABSTRACT

Effect of solid state fermentation (SSF) biomass on digestibility, nutrient utilization and rumen fermentation pattern in sheep were studied. Fourteen male adult sheep (15-18 month) were randomly divided in two groups T1 (control) and T2 (treatment). Sheep were fed ration containing 50 % jowar straw and 50% compound concentrate mixture. In vitro Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) of TMR were studied with different levels of SSF bio mass. A solid state fermentation (SSF) biomass @ 4% was added to the ration of treatment group. The nutrient requirements of sheep in term of digestible crude protein (DCP) and total digestible nutrients (TDN) were met as per ICAR (1998) feeding standards. At the end of 90 days experimental period, animals were under digestibility trial and animals of each group were used for rumen fermentation. The daily DM, CP, DCP and TDN intakes were also more or less similar in both the groups. The digestibility of DM, OM, CP, CF, EE and NFE were not affected by treatment. The rumen pH, TVFA, Total-N, ammonia-N and NPN concentrations were significantly (P<0.05) higher in treatment group as compared to control group. The protein-N concentration was higher in treatment group as compared to control group but statistically non-significant difference. The daily feed cost was Rs 5.92 and 5.87 under T_1 and T_2, respectively with non-significant difference (P>0.05).

KEYWORDS: SSF Biomass, Patanwadi Sheep, Nutrient Digestibility & Rumen Fermentation

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Original Article

INTRODUCTION

In India ruminant feeding system relies mainly on fibrous crop residues. The productivity of Indian sheep is lower than those in agriculturally developed countries. The major factors contributing to low productivity of sheep are less available nutrients from pasture and lack in adopting scientific practice of feeding, increasing intensified agriculture with good irrigation facilities and industrialization (Reddy, 1995). Forages have always provided the base upon which ruminant nutrition is built. It is evident that the ruminant animals consume grasses, leaves and stems rich in cellulose, hemicellulose and lignin. These animals do not produce the enzymes responsible for degradation of lignocelluloses but are dependent on associated microbial populations (Morgavi *et al*., 2001).

Large quantities of biologically active enzymes as animal feed additives are now produced at low cost since recent improvements in fermentation technology and biotechnology. Fibrolytic enzymes have been used to improve the nutritive value by breaking ligno-cellulose bonds in low-quality roughages and thus enhance feed colonization by increasing the numbers of ruminal fibrolytic microbes (Morgavi *et al*., 2000).

Solid-state fermentation (SSF) is defined as the fermentation involving solids in absence (or near absence)

of free water. SSF offers numerous opportunities in processing of agro-industrial residues. This is partly because solid-state processes have lower energy requirements, produce lesser wastewater and are environmental-friendly as they resolve the problem of solid wastes disposal (Pandey, 2003). Solid state fermentation holds tremendous potential for the production of enzymes by microbial flora. It is of special interest as this process includes crude fermented products that can be used directly as enzyme source (Pandey *et al*., 1999). The proposed study aims at Solid State Fermentation of crop residues and its use to improve rumen fermentation, nutrient utilization and digestibility in sheep.

MATERIALS AND METHODS

The present study on adult sheep was conducted at Animal Nutrition Research Department, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, The *Jowar* straw + *Amul dan* (concentrate mixture) based Total Mixed Ration (TMR) was fed to sheep to study the effect on voluntary feed intake, nutritional status, nutrient utilization and rumen fermentation pattern.

Experimental Animals

Fourteen adult sheep of 15 – 18 months were randomly divided into two groups with average body weight of 27.66 kg. The selected sheep were divided into two groups T1 (control) and T2 (treatment) of seven in each. The details regarding experimental sheep selected for the experiment are presented in Table 1.

Plan and Design of Experiment

In vitro Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) of TMR with different levels of SSF bio mass were studied. The TMR was prepared using jowar straw and Amul dan in the ratio of 50:50. The SSF bio mass with known activity of carboxymethylcellulase (3000 IU/g) and xylanase (1500 IU/g) (procured from Department of Microbiology, Gujarat vidhyapith, sadra) was incorporated at 0, 1, 2, 3, 4, 5, 6, 7% level in TMR. The SSF biomass at 4% level gave the highest *in vitro* digestibility hence, was used for *in vivo* study (Table:3). All the ingredients used in preparation of TMR including *jowar* straw and Amul Dan were purchased in bulk for the study period.

Feeding and Management of Experimental Sheep

The sheep under control group were fed TMR without SSF biomass where as sheep under treatment group were fed TMR as that of control group plus SSF biomass. Individual feeding of all the sheep was carried out during the study period. Quantity of TMR offered was adjusted at weekly interval according to change in body weight of sheep. The quantity of TMR required each day was offered in two installments *i.e.* half in morning and half in evening. The nutrient requirements of sheep in terms of DCP and TDN were met as per ICAR (1998) feeding standards. The sheep were housed in sheds with proper ventilation, flooring and tying arrangements with facility of individual feeding. The sheep were let loose daily (except during the period of digestion trial) in an open paddock, for two hours in the morning from 8.30 to 10.30 a.m. and one hour in the afternoon from 4.00 to 5.00 p.m. under controlled conditions for exercise. During these periods, they had free access to fresh, clean and wholesome drinking water. The leftover of TMR for individual sheep was recorded daily in the morning after 8 a.m. throughout the experimental period. Sheep were vaccinated for PPR and enterotoxaemia and deworming of all sheep with broad spectrum anthelmintics was also carried out before start of experiment. The sheep were adopted for respective feed for fifteen days. The experimental sheep were weighed at weekly interval in the morning between 8.00 to 8.30 a.m. for three consecutive days before feeding and watering for the entire experimental period of 90 days.

Digestibility Trial

The digestion trial was conducted on all the animals at the end of experimental period. The collection period was of 5 days during which the representative samples of TMR along with the left over and faeces voided were collected.

Analysis of Feed Samples

Daily representative samples of TMR fed to the sheep along with the left over and faeces were collected during digestibility trial and the pooled samples collected during the digestion trial were analyzed for proximate principles as per AOAC (1995).

Collection and Analysis of Rumen Liquor

Rumen liquor samples were collected from four sheep in each treatment once at the end of experiment. The samples were collected at 0 hr (pre feeding) and at 3 and 6 hrs post feeding to study progressive changes in the pH, total volatile fatty acids (TVFA) and various nitrogenous constituents of strained rumen liquor (SRL). About 75 ml of rumen liquor was collected from each sheep using stomach tube and employing suction (Lane *et al.*, 1968). The rumen liquor was immediately brought to the laboratory and strained through four layers of cheese cloth. The pH of SRL was determined using “Systronic” digital pH meter having glass and reference electrodes. The total volatile fatty acids were determined by Markham steam distillation method (AOAC, 1995). Ammonia nitrogen was estimated as per Conway (1957) method immediately from SRL. However, total-N was determined by Kjeldahl’s method. Rest of the SRL was stored in glass bottles by adding mercuric chloride @ 0.5 g / 100 ml SRL and preserved in refrigerator for further analysis. Non protein nitrogen was estimated from SRL following Kjeldahl’s method after precipitating the protein with 20% trichloro acetic acid solution. However, protein nitrogen content was calculated by difference of total nitrogen and non-protein nitrogen. Observations of various parameters recorded during experimental period were tabulated and the data generated were analyzed statistically as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSIONS

Nutrient Intake

The dry matter intake (DMI) in g/d and % BW between two groups was similar suggesting that the SSF biomass did not affect the dry matter intake (Table 4). The organic matter intake (g/d and % BW) of sheep had also shown non-significant ($P>0.05$) difference (Table 4). In accordance to present result, many workers have reported same finding. Singh and Das (2009) reported DMI 542 and 530.2 g/d in kids fed TMR (50:50) with or without enzymes respectively with non-significant difference ($P>0.05$). Titi and Lubbadeh (2004), Almaraz *et al.* (2010) observed no change in DMI by enzyme supplementation in lambs. Singh and Das (2008) observed 3.35 and 3.41 % DMI in goat fed with TMR (50:50) with or without enzymes, respectively with non-significant difference. Oza *et al*. (2015) also reported no significant effect of SSF feeding on growth performance in Surti kids. The values for CP, DCP and TDN intakes were statistically similar in both the groups (Table 4).

Rumen Metabolites

Data illustrated in Table 5 indicated that ruminal pH were significantly lower in enzyme treated group then control ($P>0.05$). Similarly, significantly lower level of ruminal pH found in enzyme treated group (6.71,6.45 and 6.47) then control (6.81) while salman *et al*. (2008) studied effect of fungus treatments (Trichoderma viride) fermented with

sugar beet pulp was supplemented at 0.3, 0.6 and 0.9% (w/w) to complete feed mixture in goats.. The decline in rumen pH observed with SSF supplementation may be attributed to increased TVFA concentration. On the contrary, Pinos-Rodriguez *et al*, (2008) reported that rumen pH 6.42 and 6.28, 6.58 and 6.57 & 6.69 and 6.65 in control and supplemented group, respectively for the diets with three F:C ratio (40:60, 50:50 and 60:40) fed to the lambs with no significant difference. Contrary to present findings, Singh and Das (2008) in goat, Judkins and Stobart (1988), Lee *et al.* (2000) and Avellaneda *et al*. (2009) in lambs observed that rumen pH was not affected by enzyme supplementation group (P>0.05) in to basal diets.

The average value of TVFAs, total-N, ammonia-N and NPN concentrations were significantly affected in treatment group as compared to control group (P>0.05). However, the average values of protein-N concentrations were numerically higher in treatment group as compared to control group (Table 3). The present findings are in agreement with Salman *et al.* (2008) for significantly higher TVFAs, total-N, ammonia-N and NPN concentrations were found in goat fed complete feed with fermented sugar beet pulp at different levels then control. Higher level of TVFAs shows the anaerobic fermentation of enzyme treated group (T2) was more efficient faster yielding more TVFA's than that in control groups. This could be result of higher availability of fermentable soluble carbohydrates due to increased fibrolytic activity in rumen. Values of ammonia-nitrogen were higher (P<0.05) in SSF treated T2 group than that of T1. These results indicated that the release of ammonia-nitrogen from those ration were easier than control rations, or that treated ration were it is well utilized by rumen microbes. Gado *et al.* (2011) observed that the TVFAs were significantly higher (P<0.05) in enzyme supplemented group (9.32 meq/dl) as compared to control group (7.78 meq/dl) in lambs. Singh and Das (2008) conducted an experiment to evaluate the effect of fibrolytic enzyme treatment of oat hay on rumen fermentation and nutrient utilization in goats and ammonia nitrogen and total nitrogen concentration were not affected by the enzyme treated group however TVFAs concentration was significantly higher (P<0.05) in enzyme treated group (159.40 m mol/l) as compared (147.90 m mol/l) to control. Patel *et al*. (2015) reported no effect of fibrolytic enzyme supplementation on average value of pH, TVFA and ammonia-N concentrations. However, the total-N and protein-N concentrations were significantly higher in treatment group as compared to control group in sheep.

Digestibility of Nutrients

Nutrient digestibility coefficients and nutritive value of the experimental rations are shown in Table 4. The digestibilities of various nutrients DM, OM, CP, CF, EE and NFE were not affected in enzyme supplemented group. However, the crude protein digestibility was less in T2 then T1 (P<0.05). Similar to present result, Patel *et al*. (2015) found no effect of digestibilities of nutrients DM, OM, CF and EE by fibrolytic enzyme supplementation in sheep However, digestibilities of CP and NFE were significantly higher in fibrolytic enzyme supplemented group.Pinos-Rodriguez *et al*. (2008) and Avellaneda *et al.* (2009) in sheep observed non-significant difference in DM digestibility (P>0.05) for groups with or without exogenous fibrolytic enzymes. Awawdeh and Obeidat (2011) reported that CP digestibility was 73.4 % and 71.9 % respectively under control and treatment groups with statistically non-significant difference for lambs fed fibrolytic enzyme to the basal diet. Similarly, most of the researchers reported that no effect of fibrolytic enzyme supplementation on digestibilities of nutrients (Avellaneda *et al.* 2009, Giraldo *et al*. 2008, Gonzalez *et al*. 2008, Muwalla *et al.* 2007), In contrary, Singh and Das reported the digestibility of DM, OM, CP and NDF were higher in treated group (72.61%, 74.95%, 70.98% and 62.42%) as compared to control group (68.03%, 71.31%, 65.94% and 62.06%). Bala *et al.* (2009) found significant improvement (P< 0.05) in the digestibility of DM, OM, CP, NDF, ADF and total carbohydrates due to enzyme supplementation in goats. Enzyme supplementation improve nutrient digestibility in kids

(Singh and Das 2009), in lambs (Titi and Tabbaa 2004), in goat (Ramli *et al.* 2005 and Salman *et al.* 2008). Supplementation of enzyme (with different compositions) has been utilized to improve diet digestibility and productive efficiency of ruminants. However, the improvements in animal performance have been inconsistent and variable (Beauchemin *et al.* 2003). Several factors contribute to such variability and inconsistency including the composition of enzyme, inclusion level, inclusion method (i.e., to which dietary ingredient), time of inclusion (i.e., just before feeding or pre-incubation), experimental conditions (i.e., dietary composition and degree of energy limitation in the basal diet), production level of animals, and other factors (Awawdeh and Obeidat 2011 and Beauchemin *et al*. 2003).

CONCLUSIONS

In present study, we found higher DMD and OMD in different level of SSF supplemented group in *in vitro* but we failed to achieve same result in body of sheep when SSF added at 4% level in feed for that might be some factor need to be evaluated i.e. level of supplementation, method of inoculation, level of concentrate: forage ratio for that further more studied needed. However, SSF biomass supplementation significantly improved rumen fermention in sheep.

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APPENDICES

Table 1: Grouping of Experimental Sheep

Sheep No.	Initial B. Wt. (kg)	Sheep No.	Initial B. Wt. (kg)
T_1 (Control)		T_2 (Treatment)	
1	29.67	8	18.50
2	28.83	9	24.00
3	26.17	10	29.33
4	35.67	11	31.67
5	30.00	12	29.00
6	18.67	13	30.50
7	26.83	14	28.69
Average ± SE	**27.95 ± 1.94**	**Average ± SE**	**27.38 ± 1.74**

Table 2: Average Proximate Composition* (% on DM Basis) of Feeds, Fodder and Total Mixed Ration used under Experiment

Feed	Jowar Straw	Concentrate Mixture	TMR
CP	4.36±0.13	18.25±0.29	11.01±0.03
EE	2.23±0.06	3.01±0.06	2.78±0.03
CF	32.48±0.29	12.31±0.05	20.78±0.10
NFE	52.79±0.51	53.48±0.15	53.22±0.64
Ash	8.11±0.35	12.61±0.12	12.19±0.58
Silica	2.75±0.10	2.72±0.06	2.79±0.04
Ca	0.56±0.02	0.66±0.01	0.67±0.01
P	0.32±0.01	1.26±0.01	0.68±0.02

* Average of four samples

Table 3: *In vitro* DMD (Dry Matter Digestibility) and OMD (Organic Matter Digestibility) of TMR (Total Mixed Ration) with Different Levels of SSF Bio Mass

Sr. No.	Sample Name	DMD%	OMD%
1	TMR+S.S.F.- 0%	55.39	56.21
2	TMR+S.S.F.- 1%	59.62	60.64
3	TMR+S.S.F.- 2%	61.78	62.59
4	TMR+S.S.F.- 3%	59.03	60.22
5	TMR+S.S.F.- 4%	64.41	65.42
6	TMR+S.S.F.- 5%	59.20	59.77
7	TMR+S.S.F.- 6%	61.32	63.18
8	TMR+S.S.F.- 7%	59.73	60.59

Table 4: Effect of SSF Biomass on Dry Matter Intake, Nutrients Intake, Digestibility in Sheep

Parameter	Control Group (T_1)	Treatment Group (T_2)	P value
DMI (g/d)	583.08±29.21	578.50±26.17	0.909
DMI (Kg/100kg)	2.10±0.04	2.10±0.03	0.921
Nutrients Intake			
OMI(g/d)	569.42±23.75	571.82±24.57	0.945
OMI (Kg/100kg)	1.87±0.04	1.90±0.04	0.647
CP (g/d)	58.77 ± 2.94	58.31 ± 2.63	0.909
DCP (g/d)	35.26 ± 1.76	34.98 ± 1.58	0.909
TDN (g/d)	338.47 ± 16.97	335.77 ± 15.19	0.907
Digestibility (%)			
DM	60.77 ± 1.61	58.66 ± 1.26	0.339

Table 4: Contd.,			
OM	65.40 ± 1.43	62.42 ± 1.15	0.132
CP*	81.81 ± 0.43[a]	79.22 ± 0.70[b]	0.009
CF	60.92 ± 0.93	59.79 ± 1.05	0.442
EE	70.47 ± 0.85	70.25 ± 1.07	0.871
NFE	63.55 ± 2.03	59.43 ± 1.41	0.122

*=P<0.05

Table 5: Effect of SSF Biomass on Rumen Fermentation Pattern in Sheep

Parameter	Control (T1)	Treatment (T2)
pH*	6.01 ± 0.25[a]	5.78 ± 0.17[b]
TVFA (mEq/L)*	126.96 ± 14.47[b]	134.92 ± 8.41[a]
Total-N (mg/dl)**	79.68 ± 6.87[b]	103.95 ± 7.41[a]
NPN (mg/dl)**	62.53 ± 4.87[b]	81.20 ± 6.62[a]
Ammonia-N** (mg/dl)	14.53 ± 3.25[b]	18.00 ± 3.20[a]
Protein-N (mg/dl)	17.15 ± 2.05	22.75 ± 0.81
Cost of feeding (Rs/animal/day)	5.92 ± 0.30	5.87 ± 0.26

a,b: values with different superscripts in a raw differ significantly

*=P<0.05; **=P<0.01